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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 1/16

A1

(11) International Publication Number: WO 98/37172

(43) International Publication Date: 27 August 1998 (27.08.98)

(21) International Application Number: PCT/US98/03218

(22) International Filing Date: 18 February 1998 (18.02.98)

(30) Priority Data:
08/802,773 21 February 1997 (21.02.97) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: DIETARY SUPPLEMENTATION WITH, AND METHODS FOR PREPARATION OF YEAST-DERIVED SELENIUM SALTS

(57) Abstract

The present invention solves the need for non-toxic forms of selenium which is an essential part of the human diet. This invention provides novel dried-yeast products containing selenium as well as a method of producing the dried yeast products. The method uses selenium having high biological activity but low toxicity. The invention also provides nutritional supplements containing the novel selenium-containing dried yeast products and methods of administering these products and supplements to improve human health.

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DIETARY SUPPLEMENTATION WITH, AND METHODS FOR PREPARATION OF YEAST-DERIVED SELENIUM SALTS

BACKGROUND OF THE INVENTION

Cross-Reference to Related Application

This application is a continuation-in-part of U.S. Patent Application Serial No. 08/719,572, filed September 25, 1996 and herein fully incorporated by reference.

1. Field of the Invention

This invention relates to the field of human dietary supplements, and more specifically to improved supplements comprising organoselenium complexes derived from yeast.

2. Background

The physiological assimilation of an adequate quantity of heavy metals is essential to human health. Failure to ingest and absorb the necessary amounts of such metals can lead to improper functioning of the body's metabolic processes, and to various diseases and disorders.

Many traditional nutrients, such as the vitamins and minerals for which the U.S. Food and Drug Administration (FDA) has established a Recommended Daily Allowance (RDA), may be consumed in large quantities without adverse health effects. However, for some essential nutrients, such as certain metallic nutrients like selenium, ingestion of high levels may have adverse health ramifications. To obtain

the needed physiological benefit of such compositions, one must balance the need for a minimum amount of such compounds with the need to protect against overingestion to the point of toxicity. Hence, for these compounds, ingesting low doses confers a significant nutritional benefit whereas at higher levels the nutritional benefit may not be realized and the potential for toxic effects exists.

Nutritionally beneficial quantities for daily doses for selenium have been found to be small. Nutritional selenium levels have been established by the FDA (see 21 C.F.R. 101.9(c)(8)(iv), January 1994). The FDA has adopted Reference Daily Intakes (RDIs) of 70 micrograms for selenium. Selenium dosage of 600 micrograms per day has been reported as safe. [Ferris G.M. Lloyd, et al., App. Clin. Biochem., 26:83-88 (1989)]. At about this dosage, glutathione reductase converts selenogluthatione to hydrogen selenide in the liver and erythrocytes and is ultimately excreted. Thus, at this dosage, the body is able to safely metabolize and excrete selenium.

Humans and animals can metabolize both inorganic and organic forms and convert non-methylated selenium to mono-or-di-or trimethylated derivatives, of which the monomethylated derivatives are most toxic. [Bedwal, R.S., et al., Medical Hypotheses, 41(2):150-159 (August 1993)].

There are several potential health benefits resulting from the ingestion of low levels of selenium. For example, it is understood that sodium selenate (an inorganic form of

selenium) at low concentrations works in conjunction with vanadium to improve glucose tolerance and increase the levels of glucose-induced insulin release, whereas at higher levels these effects are reversed. [Furnsinn, C. et al., Internat'l J. of Obesity and Related Metab. Dis., 19(7):458-463 (1995)].

In addition, selenium is believed to reduce the risk of certain cancers, chiefly due to its properties as a strong antioxidant. [Schrauzer, G., Inorg. and Nutr. Aspects of Cancer, p. 330 (New York: Plenum Press (1978))]. Free radical antioxidant "scavengers" such as selenium are believed to react with oxidants so that the oxidants are not available to form oxidized low density lipoprotein (O-LDL), thus lowering the risk of arterial plaque deposits in blood vessels. Arterial plaque is precipitous material formed chiefly of oxidized low density lipoprotein (O-LDL). The buildup of plaque in the form of O-LDL in the arteries is understood to be a factor in ischaemic heart disease. Free radical oxidants, many of which come from naturally occurring sources such as sun exposure, metabolism of certain nutrients, and exercise, act to oxidize low density lipoprotein (LDL) into its deleterious form, O-LDL. Hence, free radical "scavengers" such as selenium are believed to react with these oxidants so that they are not available to form O-LDL, thus lowering the risk of arterial plaque deposits in blood vessels. In contrast, high density lipoprotein (HDL) is understood to have beneficial health effects in the body. HDL is understood to be a more

soluble form of lipoprotein, and its presence is not known to significantly contribute to the formation of arterial plaque. Since selenium functions to reduce the levels of O-LDL and thereby increase the level of HDL in the body, it is understood to decrease the likelihood of cardiovascular disease as well.

Most recently, a clinical study of more than 1,300 people found that those who took a daily supplement of selenium cut their overall cancer risk by nearly 40%. [Terence Monmaney, Selenium May Fight Cancer, Study Shows, LOS ANGELES TIMES, December 25, 1996, at A 1, A 29]. In addition, U.S. Patent No. 4,599,234 teaches that a combination of a selenium species (either organic or inorganic forms) with beta-carotene and a hydroxytoluene source significantly reduced the mortality of mice that were fed carcinogens and that these effects were better than those observed for the mice that were administered either selenium or beta-carotene or hydroxytoluene. U.S. Patent No. 4,564,634 teaches a selenium-based nutritive composition having antineoplastic activity in which the selenium compound is used is a novel form of selenium prepared by a reaction of selenium metal with Tung oil (9,11,13-octadecatrienoic acid).

Yeast-derived forms of selenium have been shown to be the least toxic forms of selenium, and thus a preferred source of selenium composition for human consumption. The selenium produced by yeast is biosynthesized, a conversion of inorganic selenium salts to an organic form via

incorporation in yeast. These biosynthesized selenium derivatives are better nutritive sources of selenium since they are less toxic and more easily metabolized by the mammalian system than their inorganic counterparts. A method of producing selenium-enriched food yeast such as Saccharomyces cerevisiae or Candida utilis has been reported. When dried and fed to rats, these selenium-enriched yeast effectively prevent hepatic liver necrosis. [Reed et al., Yeast Tech., AVI Publ. Co., Conn. (1973)]. Unfortunately, this method results in the production of yeast having low selenium content as well as a relatively high extracellular concentration of inorganic selenium. U.S. Patent No. 4,530,846 describes a method for producing a selenium-enriched yeast that yields yeast with a moderate intracellular selenium content of about 1,000 ppm. The yeast produced by this method are cultivated using a procedure that involves incremental feeding of the yeast culture.

There remains a need in the art for a method to produce selenium-enriched yeast that results in: (1) a high growth rate of selenium-enriched yeast; and (2) selenium-enriched yeast with high intracellular selenium content.

SUMMARY OF THE INVENTION

The present invention overcomes the shortcomings of the prior art by providing a method for producing a composition of highly active nutritional selenium, where (1) the selenium source of the compositions of the present

invention is the natural form of biosynthesized selenium; (2) the biosynthesized selenium is entirely metabolizable by the human system, and is substantially free of toxic substances; and (3) the process can be carried out efficiently and meets requirements important for commercial production.

Therefore, an object of the present invention is to provide a method for preparing biosynthesized selenium-yeast with high selenium activity and low toxicity.

It is a further object of the present invention to provide a method for the production of biosynthesized selenium having high nutritional value and low toxicity.

Another object of the present invention is to provide an improved synthetic form of nutritional selenium that is substantially similar to the naturally occurring selenium complexes found in selenium rich foods.

It is another object of the present invention to provide a method for the production of selenium-enriched yeast, where the source of the yeast for metabolizing selenium substrate is the yeast strain Saccharomyces boulardii sequela PY31.

It is a further object of the present invention to provide a form of selenium species that is essentially non-toxic to the human body.

It is another object of the present invention to provide for methods of administering yeast-derived selenium to promote good nutritional health.

It is a further object of the present invention to provide nutritional supplements that incorporate the selenium-enriched yeast produced by these methods.

Another object of the present invention is to provide a form of selenium that is essentially non-toxic to the human body and, when used as a food supplement, reduces that susceptibility of the human body to chronic disorders.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods of cultivating yeast using selenium compounds resulting in a dried selenium-enriched yeast product with high biological activity, nutritional supplements comprising this dried yeast product, as well as uses of such dried yeast product to supplement the human diet.

The process for preparing the selenium-enriched yeast product that has a high intracellular content of organically bound trivalent selenium in a highly biologically active and non-toxic form comprises the steps of:

(1) preparing an aqueous mixture of yeast growth nutrients (aqueous media);

(2) preparing an aqueous solution of selenium salt in distilled water by dissolving the selenium salt in warm distilled water and then filtering the resulting selenium solution;

(3) adding the selenium solution to the yeast growth nutrients and mixing to form a selenium growth mixture;

(4) adding the selenium growth mixture, preferably by incremental addition, to a live yeast culture to form a selenium yeast growth solution and incubating with gentle shaking action or stirring;

(5) recovering and concentrating the yeast cells from the selenium yeast growth solution;

(6) washing the recovered yeast cells to remove extracellular selenium; and

(7) pasteurizing and/or drying the washed yeast cells.

Growth media that can be used in the first preparing step of present invention include 25° Brix molasses [TCT, Gold Coast], 38° Brix molasses [TCT, Gold Coast], glucose media, and potato dextrose broth. In addition, Brix molasses with a higher sugar content, e.g., 79° Brix [TCT, Gold Coast], may be used and then diluted with distilled water to form a Brix solution of lower sugar content. Numerous other growth media that are known to support the growth of yeast from the Saccharomyces family may be used and could be readily selected by one of skill in the art. In a preferred form, a mixture of different growth media may be used.

In addition, numerous vitamins and minerals may optionally be added to the yeast growth media. Such vitamins and minerals are selected from those known in the art to help sustain proper yeast growth, including but not limited to biotin, vitamin B_1, vitamin B_6, calcium

pantothenoate, inositol, copper, copper sulfate, zinc, zinc sulfate, iron, and iron sulfate.

The second preparing step in the process for producing the selenium-enriched yeast of the present invention involves preparing a selenium solution by dissolving selenium salt in distilled water and filtering the resultant selenium solution. The selenium may be in the form of an amorphous solid or an organoselenium compound. In a preferred form, sodium selenite may be used. In a preferred form, a cellulose acetate filter [Corning Scientific Co.] may be used. The resultant filtered selenium solution has between about 100 ppm and 40,000 ppm selenium.

The first addition step involves adding the selenium solution to the yeast growth nutrients (aqueous media) to form a selenium growth mixture. Once the selenium solution is added, the selenium and media may be mixed by gentle shaking action or stirring for between about 1 and about 30 minutes.

In the second addition step, the selenium growth mixture is added to live yeast cells to make a selenium yeast growth solution having selenium levels between about 100 ppm and about 20,000 ppm selenium, preferably between about 200 ppm to about 10,000 ppm, and most preferably between about 250 ppm to about 1,500 ppm of selenium. The second addition step preferably involves adding the selenium growth mixture to the yeast culture incrementally. This second addition step preferably takes place under a

controlled pH of from about 4.2 to about 6.0, and preferably from about 4.5 to 5.3. This second addition step also preferably takes place at a temperature from about 20°C to about 35°C, and preferably about 28°C to about 32°C.

The yeast employed in the second addition step preferably a food grade or edible yeast, and most preferably Saccharomyces boulardii sequela PY31. Other yeast which can be used include Saccharomyces Cerevisae or Saccharomyces Torula.

As stated above, the present invention may also employ a newly isolated and purified strain of yeast, Saccharomyces boulardii sequela PY31. Specifically, Saccharomyces cerevisiae and Saccharomyces boulardii sequela are of the same genus, and Saccharomyces boulardii sequela is described as a synonym of Saccharomyces cerevisiae. [Barnett et al., Yeasts: Characteristics and Identification, Cambridge Univ. Press (1990)]. More particularly, the novel yeast strain Saccharomyces boulardii sequela may be isolated from raw soil samples, and cultivated to yield quantities of yeast at a scale sufficient for developmental research and for production of commercial products. The novel strain of yeast, Saccharomyces boulardii sequela PY31, has been deposited in an International Repository in accord with the Budapest Treaty and has been assigned ATCC No. 74,366. This novel yeast strain is described in co-pending application Serial No. 08/719,572 filed on September 25, 1996.

Specifically, the method for isolating this novel yeast strain, Saccharomyces boulardii sequela PY31, comprises:

(1) identifying a location for collection of a soil sample, which is proximal to a germanium mine (i.e., within 100 yards of a germanium mine);

(2) sampling the soil by removing about 200 g from a depth of 5 cm to 20 cm, and transporting the sample using a sterilized bag;

(3) growing the living material on three different mediums which support the growth of all yeast, and that selectively kills bacteria without killing the yeast;

(4) separating the yeast from other living matter and then repeating this process until yeast can be grown without bacterial contaminants;

(5) selecting and restreaking the yeast colonies, and repeating this process three times;

(6) selecting the yeast colonies most vital for growth in a medium enriched with germanium;

(7) growing each selected colony on malt extract agar or dextrose agar, and selecting which colonies appear most robust, and;

(8) cultivating the selected yeast by growing 1 - 2 slants of the yeast for about 2 days at about 30°C and then transferring to the cultivated yeast about 100 mL of malt extract broth and then incubating at about 30°C for 8 - 10 hours, then

adding to the incubated mixture about 500 mL of malt extract broth and then growing the resulting mixture at about 30°C for about 6 to about 14 hours.

The present invention teaches a use of this novel yeast strain, Saccharomyces boulardii sequela PY31, to prepare selenium-enriched yeast forms according to the method described herein.

As part of the second addition step, the selenium yeast growth solution is incubated to induce yeast growth. The incubation may occur with shaking or stirring at about 200 rpm for a period of about 5 hours to about 75 hours, preferably from about 15 hours to about 60 hours, and most preferably about 20 hours. This incubation occurs at a temperature of about 25°C to about 30°C, and preferably about 30°C.

The yeast cells are then isolated from the selenium yeast growth solution by centrifuging the selenium yeast growth solution, and isolating the yeast cells. In a preferred form, the centrifugation step may occur at about 3,900 rpm.

The isolated yeast cells are then washed to remove extracellular selenium. The washing step may involve washing the isolated yeast cells between 2 and 20 times with aqueous solvent, such as a buffered aqueous solution that optionally contains chelating agents such as EDTA.

Lastly, the yeast cells are pasteurized and/or dried to produce a dried yeast product. The pasteurization step

may occur at between about 30°C and about 110°C, preferably at about 60°C. The resulting dried yeast product contains from about 300 ppm to about 6,000 ppm intracellular selenium.

The present invention also relates to the use of the dried selenium-enriched yeast products as dietary supplements. To prepare the yeast compositions of the invention for use as a dietary supplement, the dried yeast product is combined as the active ingredient in intimate admixture with a suitable carrier according to conventional compounding techniques. This carrier may take a wide variety of forms depending upon the form of preparation desired for administration, e.g., oral, sublingual, nasal, or parenteral.

In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed. For oral liquid preparations (e.g., suspensions, elixirs, and solutions), media containing for example, water, oils, alcohols, flavoring agents, preservatives, coloring agents and the like may be used. Carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like may be used to prepare oral solids (e.g., powders, capsules, pills, and tablets). Controlled release forms may also be used. Because of their ease in administration, tablets, pills, and capsules represent advantageous oral dosage unit forms, in which cases solid pharmaceutical carriers are obviously employed.

If desired, tablets may be sugar coated or enteric coated by standard techniques.

For parenteral products the carrier will usually comprise sterile water, although other ingredients may be included, e.g., to aid solubility or for preservation purposes. Injectable suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents, adjuvants, and the like may be employed.

A composition of the present invention is generally effective when parenterally administered in amounts ranging from about 1 mg of dried yeast per dose (1 dose per body weight of about 75 kg) to about 200 mg/dose of composition. A preferred amount is from about 10 mg/dose to about 50 mg/dose, and most preferred at about 20 mg/dose. This dosage of the composition translates to an amount of from about 1 μg/dose to about 200 μg/dose of selenium, preferably from about 10 μg/dose to about 50 μg/dose of selenium, and most preferably about 20 μg/dose of selenium. When orally administered, the compositions of the present invention are generally effective in approximately the same amounts as the parenteral products. Activity at this level makes the compositions particularly well suited for formulations in tablet size for oral administration. The above dosage ranges are likely to be administered at varying periods for humans, for example, from daily administration to administration at least 5 times per week. However, ultimately, the dosage regimen will depend upon

the particular needs of the user. A preferred dosage regimen for humans is 1 - 2 doses per day.

The following examples are illustrative only and do not limit the invention in any fashion.

EXAMPLE 1

The growth medium was prepared as follows. 79° Brix molasses (1000 g) from TCT, Gold Coast was diluted to 1 L with distilled water resulting in a 32° Brix solution. Then, 4.12 g of KCl was added, followed by 4.12 g of $MgSO_4.7H20$ and then 44.4 g of NH_4HPO_4, and the resulting mixture was stirred to homogeneity. To this mixture was added enough water to reach a final volume of 2L, and thus a 32° Brix molasses. The mixture was tested for sugar content by using a Brix refractometer [Cole-Palmer Instrument Co., RH13-32ATC], and the final pH was adjusted to 5.0 with HCl or NaOH. This solution was then autoclaved at 105°C for 15 minutes.

A stock solution of sodium selenate was prepared as follows. To 300 mL of distilled water at ambient temperature was added 9.0 g of sodium selenate and the resulting mixture was kept at ambient temperature for 1 hour, then filtered through cellulose acetate membrane [Corning Scientific Co].

The selenium growth mixture was prepared by adding 60 mL of the stock selenium solution to 1300 mL of 32° Brix molasses mixture (pH 5.07) and 3140 mL of water.

The yeast culture was prepared as follows. One slant of yeast were incubated for two days at 30°C, and then grown at 30°C in 200 mL of Malt extract, shaken at 200 rpm for 8 hrs, and then to this was added 300 mL of malt extract and the resulting mixture was incubated at 30°C overnight with shaking.

The yeast were cultivated as follows. 500 mL of a solution of Saccharomyces boulardii sequela PY31 was dissolved in 500 mL of distilled water and stirred at 500 rpm in a 7 L fermentation apparatus [Bioflow 2000, New Brunswick]. To this yeast culture, 4500 mL of the selenium growth mixture as added under an air flow of about 2 to about 5 L/min and over a period of about 6 hours at about 30°C. The resulting suspension was stirred for an additional 15 hours.

The resulting selenium yeast growth solution was then centrifuged at 3900 rpm for 10 minutes, and resulting yeast cream (isolated yeast cells) was washed five times with a total volume of 8 L of water.

The resulting yeast cells were dried at less than 80°C until a moisture content of 2-3% was obtained. The selenium content of the yeast was measured using atomic absorption techniques to give a yeast mass having 1839 ppm of selenium.

EXAMPLE 2

The method described in Example 1 was repeated to give a dried yeast mass having a final concentration of selenium of 1849 ppm.

EXAMPLE 3

The yeast growth nutrients were prepared as follows. 25° Brix molasses (670 g) [TCT, Gold Coast] was diluted to 1 L with distilled water, then 2.72 g of KC1 was added, followed by 2.72 g of $MgSO_4.7H20$ and then 29.28 g of NH_4HPO_4, and then enough water was added to reach a final volume of 2 L, and the resulting mixture stirred at ambient temperature until homogenous. The mixture was tested for sugar content by using a Brix refractometer [Cole-Palmer Instrument Co., RH13-32ATC], and the final pH was adjusted to 5.0. This solution was autoclaved at 105°C for 15 minutes.

A stock solution (3%) of sodium selenate was prepared as follows. To 300 mL of distilled water at ambient temperature was added 9.0 g of sodium selenate and the resulting mixture was stirred at ambient temperature for 1 hour. The resulting selenium solution was filtered through cellulose acetate membrane [Corning Scientific Co].

The yeast culture was prepared as follows. One slant of yeast were incubated for two days at 30°C, and then grown at 30°C in 200 mL of Malt extract, shaken at 200 rpm for 8 hrs, and then to this was added 300 mL of malt extract and

the resulting mixture was incubated at 30°C overnight with shaking.

A yeast growth mixture was prepared by adding 13.2 L of the selenium solution (3%) to 3989 mL of the 25° Brix molasses and 247 mL of water, and then stirred or shaken to homogeneity.

The cultivation of selenium-enriched yeast was undertaken as follows. In a 7 L fermentation apparatus [Bioflow 2000, New Brunswick] containing 70 mL of a stirred (500 rpm) yeast culture of Saccharomyces boulardii sequela PY31, 2 L of the yeast growth nutrient mixture was added over 8 hours at 30°C with stirring under an air flow of about 2 to about 5 L/min. After the addition of the yeast growth nutrient mixture, the mixture was stirred an additional 5 hours at about 500 rpm. Then, 3989 mL of 25° Brix molasses growth nutrients and 247 mL of water were added, and the resulting selenium yeast growth solution was shaken at 200 ppm for 24 hours at 30°C.

The resulting selenium yeast growth solution was centrifuged at 3,900 rpm for 10 minutes, the supernatant removed, then the yeast cells were washed once with 100 mL of EDTA (pH=7.8), once with 100 mL of 0.01 M Na_2HPO_4 buffer solution, and five times with 100 mL of distilled water.

The resulting yeast cream (isolated yeast cells) was dried in vacuo and then the selenium content of the yeast was measured using atomic absorption techniques to give a yeast mass having 4857 ppm of selenium.

EXAMPLE 4

The method of Example 3 was repeated and yielded a final dried yeast mass having a concentration of selenium of 4945 ppm.

EXAMPLE 5

A 20% glucose media was prepared by adding 400 g of glucose, with stirring, to 2 L of distilled water, followed by addition of 43 g of urea, 20 g of Na_2HPO_4, 7.6 g of $MgSO_4.7H_2O$, 4.4 g of KCl, 50 g of sodium citrate, and 10 grams of yeast extract [DIFCO, Bacto]. The resulting yeast growth nutrients were mixed and then autoclaved at 10 psi for 15 minutes.

A vitamin mixture was prepared as follows: 4 mg solid biotin, 8 mg vitamin B1, 200 mg vitamin B6, 100 mg calcium pantothenoate, and 2 g of inositol were added to 100 mL of distilled water, and the resultant solution was stirred to homogeneity and filtered through a 25 micron cellulose acetate filter [Watman].

A mineral solution was prepared as follows: 0.5 g of $CuSO4.5H_2O$, 8 g of $ZnSO_4.7H_2O$, and 3 g ferric sulfate $(Fe(SO_4)_2(NH_4)_2.6H_2O)$ were added to 1 L of distilled water and stirred to homogeneity and then autoclaved at 10 psi for 15 minutes.

A yeast growth nutrient mixture was prepared by adding 20 mL of the vitamin solution and 2 mL of the mineral solution to the glucose media.

The yeast culture was prepared as follows. One slant of yeast were incubated for two days at 30°C, and then grown at 30°C in 200 mL of Malt extract, shaken at 200 rpm for 8 hrs, and then to this was added 300 mL of malt extract and the resulting mixture was incubated at 30°C overnight with shaking.

A cultivation of selenium-enriched yeast was undertaken by adding 50 mL of 3% sodium selenate solution (as described in the above examples) to 2,500 mL of the 20% yeast growth nutrient mixture. Next, 2 L of the resulting mixture was isolated and added slowly over a period of 11 hours at 31°C to a mixture of 500 mL Saccharomyces boulardii sequela PY31 (ATCC No. 74,366) and 2 L distilled water that had been mixed to homogeneity and autoclaved at 10 psi for 15 minutes. The resulting selenium yeast growth solution was then stirred at 31°C for an additional 8 hours.

The yeast were then isolated as described in Example 1 above to yield yeast mass containing 684 ppm selenium.

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. It is also intended that the present invention cover modifications and variations of the dried yeast selenium compositions and method for using them to accomplish their claimed uses within the scope of the appended claims and their equivalents.

WE CLAIM:

1. A method for producing selenium-enriched yeast product comprising the steps of:

preparing a mixture of yeast growth nutrients (aqueous media);

preparing a selenium solution in distilled water by dissolving a selenium compound in distilled water and then filtering the resulting selenium solution;

adding the selenium solution to the yeast growth nutrients and mixing to form a selenium growth mixture;

adding the selenium growth mixture to a live yeast culture to form a selenium yeast growth solution and incubating with agitation;

recovering and concentrating the yeast cells from the selenium yeast growth solution;

washing the recovered yeast cells to remove extracellular selenium; and

drying the washed yeast cells.

2. The method of claim 1, wherein the yeast growth nutrients are selected from the group consisting of Brix molasses, glucose media, and potato dextrose broth.

3. The method of claim 1, wherein the first preparing step comprises preparing a mixture of yeast growth nutrients using a mixture of different growth media.

4. The method of claim 1, wherein the second preparing step comprises dissolving in distilled water a selenium compound selected from the group consisting of a selenium salt and an organoselenium species.

5. The method of claim 1, wherein the second preparing step comprises adding between about 100 ppm to about 40,000 ppm selenium to distilled water and filtering through a cellulose acetate filter.

6. The method of claim 1, wherein the second adding step comprises adding the selenium growth mixture to the live yeast culture incrementally.

7. The method of claim 1, wherein the yeast in the second adding step is selected from the group consisting of S. cerevisiae (Brewer's yeast), S. Torula, S. uvarum, and Saccharomyces boulardii sequela PY31 (ATCC No. 74,366).

8. The method of claim 1, wherein the second adding step comprises gentle shaking at about 20°C to about 30°C.

9. The method of claim 1, wherein the second adding step comprises incubating for about 5 hours to about 75 hours.

10. The method of claim 1, wherein the recovering and concentrating step comprises centrifugation.

11. The method of claim 1, wherein the washing step comprises washing the isolated yeast cells multiple times with buffered aqueous solvent.

12. A selenium yeast product produced by the method of any of claims 1-11.

13. A nutritional supplement comprising the selenium yeast produced by the method of any of claims 1-11 admixed with an acceptable pharmaceutical carrier.

14. A process of supplementing the human diet comprising administering an effective amount of the selenium yeast product produced by the method of any of claims 1-11.

15. A process of supplementing the human diet comprising administering an effective amount of the selenium yeast product of claim 12.

16. A process of supplementing the human diet comprising administering an effective amount of the nutritional supplement of claim 13.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/03218

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12N 1/16
US CL : 435/255.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/255.1, 435/255.2, 426/60, 426/62, 424/93.51

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAPLUS, MEDLINE, BIOSIS, AGRICOLA, WPIDS, EMBASE
search terms: selenium, yeast, Saccharomyces, product

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHRAUZER et al. Observations on Human Selenium Supplementation. Traces and Substances in Environmental Health. 1987, Vol. 13, pages 64-67, see entire document.	12-16
Y	US 4,530,846 A (NAGODAWITHANA ET AL) 23 JULY 1985, examples 1-6, col. 3, lines 23, 44, 64-66, col. 4, line 5, col. 5, lines 20-21, col. 6, lines 25 and 43-45.	1-16
Y	TALARO et al. Foundations in Microbiology. Dubuque, IA: Wm. C. Brown Publishers. 1993, pages 281-282, see entire document.	5

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search 30 MARCH 1998	Date of mailing of the international search report 12 JUN 1998
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Form PCT/ISA/210 (second sheet)(July 1992)*